THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES & TECHNOLOGY

Discovery of novel hypertrophic cardiomyopathy genes and their functional implications using multi-model approach

SYNOPSIS

BY

PRATUL KUMAR JAIN

UNDER THE MENTORSHIP OF

DR. PERUNDURAI S. DHANDAPANY

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE, BENGALURU

1) Introduction and backgroud:

Cardiomyopathies are heterogenous group of muscle disorder resulting in impaired structural and functional ability of myocardium[1]. Commonly characterized by change in its size, shape, or thickness, leading to the heart's inability to pump blood effectively. There are different types of cardiomyopathy, including hypertrophic cardiomyopathy, dilated cardiomyopathy, and restrictive cardiomyopathy. These types affect the heart in various ways, such as causing the heart muscle to weaken, enlarge, thicken, or stiffen[2,3]. Some cardiomyopathies are inherited and can run in families due to genetic differences.

Focus of my study is restricted to hypertrophic cardiomyopathy (HCM) which is genetically autosomal dominant and clinically heterogenous in nature and characterized by increase in left ventricular wall and inter-septal wall thickness, in absence of any other systemic or cardiac condition that can lead to significant enlargement of heart wall like systemic hypertension or aortic valve stenosis[3]. HCM is among the most common form of cardiomyopathy with high prevalence rate of 1:250 to 1:500 and most common cause of sudden cardiac arrest in young individuals[3]. Multiple cardiac sarcomere contractile protein mutations cause HCM[4]. Currently, 11 mutated genes usually linked to HCM include β-myosin heavy chain (first discovered) and myosin-binding protein C. Other 9 genes, including as troponin T and I, regulatory and essential myosin light chains, titin, α-tropomyosin, α-actin, α-myosin heavy chain, and muscle LIM protein, are associated with less HCM instances. With almost 400 mutations, intragenic heterogeneity enhances genetic diversity[5]. However still 50 percent of cases are idiopathic in nature with no known definitive cause[5]. So far only three non sarcomeric genes have been implicated in HCM namely γ-2-regulatory subunit of the AMP-activated protein kinase (*PRKAG2*)[6], *LAMP-2*[7] and *ALKP3*[8]. Evidently there is big void in context of non sarcomeric gene variant identification and limited knowledge regarding the genetic influence of non sarcomeric proteins in HCM. In my thesis, I will outline our discovery of three novel non-sarcomeric HCM genes and their critical regulatory mechanisms in the disease pathogenesis in three distinct chapters.

The current study titled "***Discovery of Novel Hypertrophic Cardiomyopathy Genes and their functional implications using multi-model approach"*** is divided into seven chapters. The first chapter is introduction which highlights and reviews the literature on cardiomyopathy and its different types along with the global burden of the disease as well as discusses open questions. The second chapter describes the objectives of the study highlighting the need of the study.

Chapter three deals with material and methods of the thesis. Genetics (next generation sequencing), immuno-blotting, immuno-histochemistry, confocal imaging and quantitative real time PCR etc. has been predominantly utilized to achieve the objectives of the thesis. Chapter four and five discusses the findings of the thesis with respect to discovery of three novel gene loci associated with hypertrophic cardiomyopathy. Chapter four of the thesis discussing about the identification of novel gene variant (p.G47W) in ribosomal S6 kinase beta 1 (*RPS6KB1*) through whole exome sequencing and the mechanism involved in pathogenesis. This study have been published in Journal of medical genetics in 2021[9]. Chapter five highlights the discovery of a novel gene variant in tubulin tyrosine ligase gene (p.G219S) and its implication in disease pathogenesis via perturbing redox homeostasis in patient specific induced pluripotent stem cells derived cardiomyocytes (iPSCs-CMs). Sixth chapter discusses the summary and our contribution to current status of the disease understanding and the under-explored signaling gene involvement in disease causation and pathogenesis. And chapter seven consists of bibliography.

2) Objectives:

Specific objectives of my study are:

1.1) Identification of novel gene loci through next generation sequencing (NGS) for HCM

1.2) Deciphering the key mechanisms involved in disease pathogenesis corresponding to respective variants

3) Ribosomal protein S6 kinase beta-1 gene variants cause HCM by activating S6K1/rpS6/ERK signaling :

Using the above NGS methods, we discovered identical mutations (p.G47W) in the gene encoding ribosomal protein S6 kinase beta-1 (RPS6KB1 or S6K1) in two Indian families. We further identified two heterozygous variants (p.Q49K and p.Y62H) from the UK cardiomyopathy cohort in replication association studies. These variations are absent in both region-specific controls and various population genomic datasets. Further, we detected additional S6K1 mutation (p.P445S) in an Arab HCM patient. The functional effects of these mutants were assessed in cellular models by comparing representative mutant proteins of S6K1 with the wild type. The mutated proteins activated the S6K1 and hyperphosphorylated the rpS6/ERK signaling cascades, suggesting a gain-of-function effect[9].

4) ***TTL* variant causes HCM by inducing oxidative stress in patient-specific iPSC-derived cardiomyocytes:**

We used Next Generation sequencing techniques to examine two cohorts of Indian HCM patients and their respective controls. We identified an HCM patient-carrying tubulin tyrosine ligase (*TTL*) gene variant (p.G219S) that is absent in controls. TTL is involved in post-translational modification of alpha-tubulin by adding tyrosine residue to its C-terminal tail[10]. This absence leads to an abnormal de-tyrosinated and tyrosinated tubulin ratio, affecting the contractility in cardiomyocytes[11]. Our biochemical study suggests that *TTL* mutant proteins displayed delayed activity in tubulin tyrosination compared to the wild type. We modeled the patient HCM phenotypes by generating and characterizing the *TTL* mutant patient-specific iPSCs-derived cardiomyocytes (iPSC-CMs). The iPSC-CMs recapitulated hypertrophy, contractile dysfunction, and sarcomeric disarray due to increased de-tyrosinated alpha-tubulin. Furthermore, transcriptomic analysis from iPSCs-CMs revealed perturbed redox homeostasis, specifically activating NRF2 pathways leading to oxidative stress[12].

5) **Material and Methods:**

We performed whole exome sequencing (WES) of various patients and controls of different ethnicities. For functional evaluation, in accordance with different gene variants, we have used different cell lines like HEK293T, HL-1, H9C2, including human embryonic stem cells (hESCs) derived cardiomyocytes and for modeling disease in human system, we generated and characterized patient specific induced pluripotent stem cells (iPSCs) line from patient's PBMCs and differentiated to cardiomyocytes. For transcripts levels studies quantitative real time PCR was done against gene specific primers. Immunoblotting and immunohistochemistry were employed for understanding the protein levels in accordance with the need of the study against specific antibodies. Confocal imaging was used to investigate the cell size analysis and to look for levels of certain protein types.

6) **Discussion and future perspective:**

HCM is frequently characterized as a sarcomere-related disorder, mostly because of the numerous sarcomere gene mutations that have been detected in individuals and families affected by the condition[4,5]. Significant advances have been made in understanding the impact of sarcomere mutations in cardiac hypertrophy. Advancements in this field have resulted in the

development of treatments that specifically address issues related to sarcomere dysfunction. However there has been limited knowledge in comprehending the relationship between non-sarcomeric gene variants and cardiac hypertrophy. Despite several theories suggesting non-sarcomeric pathways, the knowledge remains incomplete. To add to current knowledge, this study leads to identification of three different non-sarcomeric gene variants and their associated mechanism culminating into pathological hypertrophy. Investigating non-sarcomeric gene variations in hypertrophic cardiomyopathy is critical for gaining a more complete picture of the disease's genetic landscape. This understanding has implications for diagnostics, patient care, and the development of targeted medicines (personalized/precision medicine), all of which aim to improve outcomes for HCM patients.

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